Molecular and Cellular Endocrinology 332 (2011) 163–169

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Activities of bone morphogenetic proteins in prolactin regulation by somatostatin analogs in rat pituitary GH3 cells

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ARTICLE INFO

Article history:
Received 12 June 2010
Received in revised form 9 October 2010
Accepted 11 October 2010

Keywords:
Bone morphogenetic protein
Bromocriptine
GH3
Octreotide
Pasireotide and Prolactin

ABSTRACT

Involvement of the pituitary BMP system in the modulation of prolactin (PRL) secretion regulated by somatostatin analogs, including octreotide (OCT) and pasireotide (SOM230), and a dopamine agonist, bromocriptine (BRC), was examined in GH3 cells. GH3 cells are rat pituitary somato-lactotrope tumor cells that express somatostatin receptors (SSTRs) and BMP system molecules including BMP-4 and -6. Treatment with BMP-4 and -6 increased PRL and cAMP secretion by GH3 cells. The BMP-4 effects were neutralized by adding a BMP-binding protein Noggin. These findings suggest the activity of endogenous BMPs in augmenting PRL secretion by GH3 cells. BRC and SOM230 reduced PRL secretion, but OCT failed to reduce the PRL level. In GH3 cells activated by forskolin, BRC suppressed forskolin-induced PRL secretion with reduction in cAMP levels. OCT did not affect forskolin-induced PRL level, while SOM230 reduced PRL secretion and PRL mRNA expression induced by forskolin. BMP-4 treatment enhanced the reducing effect of SOM230 on forskolin-induced PRL level while BMP-4 did not affect the effects of OCT or BRC. Noggin treatment had no significant effect on the BRC actions reducing PRL levels by GH3 cells. However, in the presence of Noggin, OCT elicited an inhibitory effect on forskolin-induced PRL secretion and PRL mRNA expression, whereas the SOM230 effect on PRL reduction was in turn impaired. It was further found that BMP-4 and -6 suppressed SSTR-2 but increased SSTR-5 mRNA expression of GH3 cells. These findings indicate that Noggin rescues SSTR-2 but downregulates SSTR-5 by neutralizing endogenous BMP actions, leading to an increase in OCT sensitivity and a decrease in SOM230 sensitivity of GH3 cells. In addition, BMP signaling was facilitated in GH3 cells treated with forskolin. Collectively, these findings suggest that BMPs elicit differential actions in the regulation of PRL release dependent on cellular cAMP-PKA activity. BMPs may play a key role in the modulation of SSTR sensitivity of somato-lactotrope cells in an autocrine/paracrine manner.

1. Introduction

BMPs, which belong to the TGF-β superfamily, were originally identified as active components in bone extracts that are capable of inducing bone formation at ectopic sites. A variety of physiological BMP actions in many endocrine tissues, including the ovary, pituitary, thyroid and adrenal, have been discovered (Shimasaki et al., 2004; Otsuka, 2010). There is also increasing evidence that locally produced BMPs play key roles in differentiation of the pituitary. The BMP system is known to play important roles in initial development of the anterior pituitary (Scully and Rosenfeld, 2002). BMP-4 is required during the first stage of pituitary organogenesis for the proliferation of Rathke's pouch, which gives rise to Pit-1 lineage cells including lactotrope cells. During the subsequent stages of pituitary organogenesis, inhibition of BMP-2 by fibroblast growth factor (FGF)-8 leads to differentiation of corticotrope cells (Kioussi et al., 1999; Dasen and Rosenfeld, 2001). BMP-4 not only governs the pituitary organogenesis but also plays a key role in the pathogenesis of differentiated pituitary lineages (Giacomini et al., 2006; Labeur et al., 2010; Tsukamoto et al., 2010).

Abbreviations: ActRI and ActRII, activin type I and type II receptor; ALK, activin receptor-like kinase; BMP, bone morphogenetic protein; BMPRI and BMPRII, BMP type I and type II receptor; BRC, bromocriptine; DA, dopamine agonists; D2R, dopamine D2 receptor; FSK, forskolin; OCT, octreotide; SSTR, somatostatin receptor; TGF-β, transforming growth factor-β.

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doi:10.1016/j.mce.2010.10.008

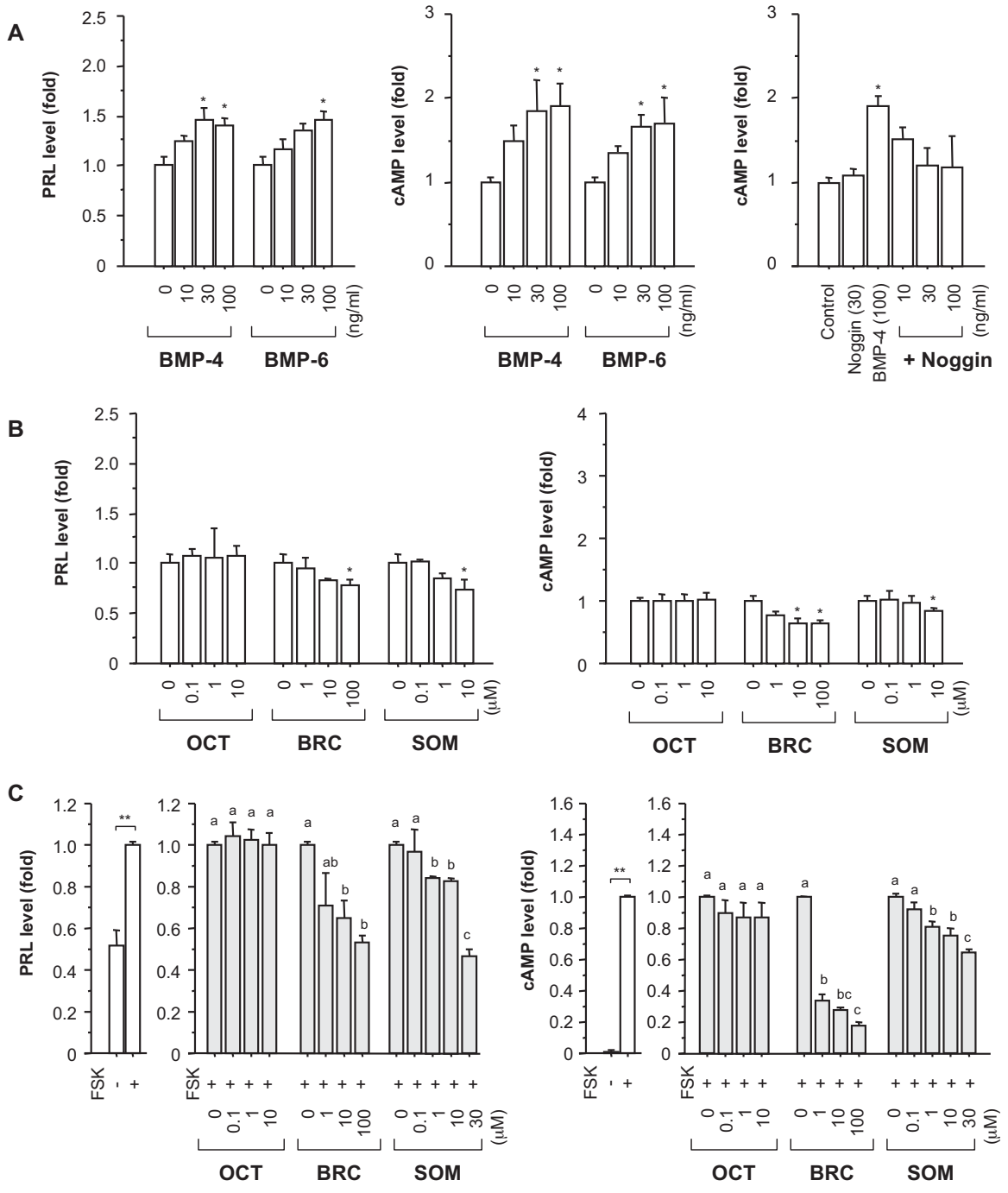

Fig. 1. Effects of BMPs and somatostatin analogs on prolactin secretion. (A) GH3 cells (1×10^5 viable cells) were precultured in serum-free DMEM/F12. The cells were then treated with BMP-4 (10–100 ng/ml), BMP-6 (10–100 ng/ml) and Noggin (10–100 ng/ml). After 24-h culture, the supernatants of culture media were collected, and prolactin (PRL) and cAMP levels were determined by specific enzyme immunoassays. For measurement of cAMP levels, cells were cultured with serum-free medium containing 0.1 mM of IBMX. (B and C) GH3 cells (1×10^5 viable cells) were precultured in serum-free DMEM/F12. Cells were treated with octreotide (OCT; 0.1–10 μM), bromocriptine (BRC; 1–100 μM) or pasireotide (SOM; 0.1–30 μM) in the absence (B) or presence (C) of forskolin (FSK; 1 μM). After 24-h culture, the culture media were collected, and PRL and cAMP levels were determined. Results in all panels are shown as mean ± SEM of data from at least three separate experiments, each performed with triplicate samples. The results were analyzed by ANOVA with Tukey–Kramer's post hoc test or unpaired *t*-test. For each result within a panel, $^*P < 0.05$ vs. control group in each panel; and the values with different superscript letters are significantly different at $P < 0.05$.

Dopamine agonists (DA) are the clinical treatment of choice for prolactin (PRL)-secreting pituitary adenomas (Casanueva et al., 2006). They control PRL secretion and cell proliferation by interacting with the dopamine D2 receptor (D2R), which subsequently activates various transduction pathways (Missale et al., 1998). D2R agonists are efficient in the majority of cases; however, some patients with prolactinomas fail to obtain PRL normalization and reduction in tumor size even with the most potent dopamine agonist, cabergoline (Molitch, 2005; Gillam et al., 2006; Hofland et al., 2010). These prolactinomas, resistant to DA, are usually large and/or invasive, and surgery therefore cannot be a complete curative treatment. In such tumors poorly or partially responsive to DA, an alternative medical treatment is needed. Experimental data have demonstrated that different somatostatin receptor (SSTR) subtypes are expressed at various levels in prolactinomas, SSTR-5 being the most important in the regulation of PRL secretion (Shimon et al.,